THE PRODUCTION OF A HYPERIMMUNE SERUM FOR INFECTIOUS ABORTION IN MARES*

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In carrying on investigations of infectious abortion in mares, it occurred to us that a prophylactic serum as a protective agent against the disease might be produced. The question arose whether Bacillus abortivo-equinus† would produce a sufficient degree of immunity in a mare for the serum to have any prophylactic properties, and whether the amount of this serum necessary for protection would not be too great for practical purposes.

An old mare was selected for the production of this serum. The animal was in good health, of medium size, and had never had any disease so far as we were able to discover. This mare had been used previously in an experiment to determine the effect of inoculation with B. abortivo-equinus. This had no effect on the production of a potent serum. The entire treatment was as follows:

Oct. 22, 1913.—The mare received by way of the jugular vein 0.25 c.c. of a 24-hour mixed-broth culture of B. abortivo-equinus, diluted in 5 c.c. of normal sterile salt solution. The temperature at the time of inoculation, 5 p. m., was 99.8; on October 23, at 9 a. m., it was 99.4.

Nov. 4.—The mare received 1.5 c.c. of culture as before. The temperature before inoculation, at 3 p. m., was 99.6. After inoculation, at 3:30 p. m., it was 99.6; at 4 p. m., 99.8; at 4:30 p. m., 100.4; at 5 p. m., 100.6; on November 5, at 9 a. m., 100.2; at 1 p. m., 102.8; and at 4 p. m., 103.4.

Nov. 12.—The mare received 3 c.c. of a 45-hour culture in 7 c.c. normal sterile salt solution. The temperature at 2:30 p. m., before inoculation, was 100. After inoculation, at 3:30 p. m., it was 101.2; at 4:30 p. m., 103.4; on November 13, at 8:30 a. m., 102.2; at 2:30 p. m., 103; on November 14, at 11 a. m., 101.6; on November 15, at 9 a. m., 99.6.

March 13, 1914.—The blood showed an agglutination titer of 1:250.

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† Our attention has been called to the fact that in previous articles the name suggested by us for the organism causing infectious abortion in mares, Bacillus abortivus equinus, is a trinomial designation contrary to the accepted rule for bacterial nomenclature. This being the case, we should suggest the name, Bacillus abortivo-equinus. The trinomial name appears in "Investigations of the Etiology of Infectious Abortion of Mares and Jennets in Kentucky," by Edwin S. Good and Lamert S. Corbett, The Journal of Infectious Diseases, 1913, 13, p. 53; in "Bacillus Abortivus Equinus as an Etiological Factor in Infectious Arthritis of Colts," by Edwin S. Good and Wallace V. Smith, The Journal of Infectious Diseases, 1914, 15, p. 347; and in "Maintenance of the Virulence of Bacillus Abortivus Equinus," by the same authors, The Journal of Medical Research, 1916, 33, p. 493.

March 23.—A mixed vaccine, which had been washed from agar slants, heated to 65 C. for 2 hours, and carbolated 0.2 of 1%, and which contained approximately 12,560,000,000 bacteria per cubic centimeter, was injected intrajugularly. The vaccine, 2 c.c., was diluted in 3 c.c. normal sterile salt solution. The temperature rose 1 degree as a result of this injection.

April 6.—Mare received 5 c.c. as before. Temperature rose only 0.6 degree.

April 19.—Eight cubic centimeters were given. Temperature rose 1 degree.

May 4.—Ten cubic centimeters were given. Temperature rose 1.5 degrees.

May 19.—Blood was drawn for an immune serum; the agglutination titer of this serum was 1:10,000.

March 6, 1915.—A series of inoculations was started, the intention being to produce a hyperimmune serum to be used in combating infectious abortion of mares. Blood was drawn; its agglutination titer was 1:100. A mixed 48-hour broth culture was given, 0.25 c.c. intrajugularly. The culture was diluted with 5 c.c. salt solution.

March 15.—Received 1 c.c. as before.

March 23.—Received 2 c.c. of a 24-hour culture in 3 c.c. salt solution.

March 31.—Received 4 c.c. as before (24-hour culture).

April 8.—Received 5 c.c.

April 16.—Received 7 c.c. The animal seemed depressed and rather emaciated. It did not seem advisable to give any more of the culture.

April 24.—The animal appeared to be in much better condition. Blood to the amount of 500 c.c. was drawn from the jugular.

April 26.—Blood to the amount of 800 c.c. was drawn from the jugular. Both these lots were allowed to clot. The agglutination titer of these samples was 1:1250. This serum was labeled "4/26".

May 15.—Mare received 7.5 c.c. culture as before.

May 26.—Blood to the amount of 800 c.c. was drawn from the jugular and defibrinated. The whole defibrinated blood was used in tests. It was labeled "5/26".

In order to determine whether this serum was of any bactericidal value, it was put to the following test,[1] the sample labeled "5/26" being used.

One cubic centimeter of the mare's blood and 1 c.c. of normal horse serum were inactivated by heating to 56 C. for one-half hour. They were then diluted 1:50. Two cubic centimeters normal fresh horse serum were then diluted 1:10. A 24-hour broth culture of B. abortivo-equinus was diluted 1:500.

One cubic centimeter of sterile broth was placed in 12 test tubes. To the 1st one of these, 1 c.c. of the diluted immune blood was added and thoroughly mixed. Of this mixture 1 c.c. was placed in the 2nd tube, 1 c.c. from the 2nd in the 3rd, and so on until the last tube was reached, from which 1 c.c. was discarded. The tubes now contained the following dilutions: 1:100; 1:200; 1:400; 1:800; 1:1600; 1:3200; 1:6400; 1:12,800; 1:25,600; 1:51,200; 1:102,400; 1:204,800.

Four tubes with normal horse serum were treated as the 12 tubes just mentioned. The dilutions in these tubes were: 1:100; 1:200; 1:400, and 1:800. Of the diluted bacterial emulsion 0.5 c.c., and of the diluted complement serum 0.5 c.c. were added to each tube.

[1] Kolmer: Infection, Immunity and Specific Therapy, p. 349.

Table 1 gives the results of the test for bactericidal activity on the part of the immune serum (A), together with the results of 8 control tests (B).

TABLE 1

A.—RESULTS OF BACTERIOLYTIC TEST OF IMMUNE BLOOD "5/26"

Tube	Dilution of Immune Blood	Mixed Bacillus abortivo-equinus, 24-hour Culture	Horse Complement	Results*
1	1: 100	0.5 c.c.	0.5 c.c.	Thousands of colonies†
2	1: 200	0.5 c.c.	0.5 c.c.	Sterile
3	1: 400	0.5 c.c.	0.5 c.c.	Several hundreds
4	1: 800	0.5 c.c.	0.5 c.c.	Several hundreds
5	1: 1600	0.5 c.c.	0.5 c.c.	About 200
6	1: 3200	0.5 c.c.	0.5 c.c.	About 250
7	1: 6400	0.5 c.c.	0.5 c.c.	About 100
8	1: 12,000	0.5 c.c.	0.5 c.c.	Less than 100
9	1: 25,600	0.5 c.c.	0.5 c.c.	Thousands
10	1: 51,200	0.5 c.c.	0.5 c.c.	Many thousands
11	1: 102,400	0.5 c.c.	0.5 c.c.	Infinite numbers
12	1: 204,800	0.5 c.c.	0.5 c.c.	Infinite numbers

B.—RESULTS OF THE CONTROL TESTS

Control	Purpose of Test	Substance Tested	Substances Used in Test	Results
1	To show the number of colonies in culture	0.5 c.c. culture	10 c.c. agar cooled to 42 C.	Infinite numbers
2	To show that the number of colonies had multiplied during incubation	0.5 c.c. culture	1.5 c.c. broth‡	Colonies denser than in Control 1
3	To show bacteriolytic properties of complement	Tube 1. 1 c.c. complement, 1:10	0.5 c.c. culture and broth enough to make 2 c.c. added to each tube	Many thousands
		Tube 2. 0.5 c.c. complement, 1:10		Infinite numbers
		Tube 3. 0.2 c.c. complement, 1:10		Infinite numbers
		Tube 4. 0.1 c.c. complement, 1:10		Infinite numbers
4	To show sterility of complement	0.5 c.c. complement	1.5 c.c. broth	No colonies
5	To show sterility of hyperimmune blood	1 c.c. immune blood 1:100	1 c.c. broth	No colonies
6	To show sterility of control serum	1 c.c. control serum 1:100	1 c.c. broth	No colonies
7	To show possible presence of complement in immune blood	1 c.c. immune blood 1:100	0.5 c.c. culture and 0.5 c.c. broth	Infinite numbers
8	To show possible presence of complement in control serum	1 c.c. control serum 1:100	0.5 c.c. culture and 0.5 c.c. broth	Infinite numbers

* In each of 4 tubes prepared with normal horse serum, instead of the immune serum, the number of colonies was infinite.

† In bactericidal experiments, the paradoxic results obtained, as in this instance, were caused by excess of amboceptors in the immune serum. Kolmer states (p. 354):[1] "In a mixture of bacteria, complements, and large amounts of amboceptor, the complement is bound not only by the amboceptors anchored to the bacteria, but also in a large measure by free amboceptors that are not anchored to bacteria. A portion of the anchored amboceptor, therefore, finds no complement at its disposal, and is therefore unable to exert any bactericidal action which gives rise to a relative lack of complement."

‡ The last 7 controls were incubated for 3 hours. All the tubes were then plated in agar, the plates incubated at 37 C. for 24 hours, and then the colonies counted.

Altho the plate in which the dilution of the immune blood was 1 : 200 was the only plate absolutely sterile, yet the bacteriolytic power of the blood was very marked in the dilutions of 1 : 400, 1 : 800, 1 : 1600, 1 : 3200, 1 : 6400, and 1 : 12,800. We consider that since the number of colonies in the dilution of 1 : 12,800 was less than 100, the titer of the serum must lie somewhere near that dilution.

Since this blood exhibited such excellent bacteriolytic power in vitro, experiments were made to see whether these effects could be shown in vivo.

In preliminary experiments, we determined that 1 c.c. of a 24-hour mixed-broth culture of B. abortivo-equinus subcutaneously administered, aborted a guinea-pig in 5 days, and that 0.1 c.c. culture given intravenously, was the lethal dose for rabbits in from 3 to 4 days.

On April 29, 1915, Rabbit 194 received 0.5 c.c. of a 24-hour mixed-broth culture of B. abortivo-equinus intravenously, and 3 c.c. of the hyperimmune serum "4/26" subcutaneously. Death occurred on May 5, 1915.

On April 29, Rabbit 192 received 0.5 c.c. of the culture intravenously, and 5 c.c. of the serum "4/26" subcutaneously. This animal developed marked symptoms, stopped eating, had decided conjunctivitis, but recovered and was normal again on May 17. A slight congestion occurred at the seat of inoculation on the margin of the ear, but this wound soon healed.

On April 29, Rabbit 199 received 0.2 c.c. of the culture intravenously, and 10 c.c. of the serum "4/26" subcutaneously. This animal died on May 3.

Five-tenths cubic centimeter of the culture represented 5 times the lethal dose. With the following animals this dose was cut to 0.1 c.c., with more satisfactory results, altho 0.1 c.c. had previously been shown to be extremely pathogenic, killing in from 3 to 4 days.

Rabbit 178 received 0.1 c.c. of a 24-hour mixed-broth culture intravenously, and 3 c.c. of the hyperimmune serum "4/26" subcutaneously. This rabbit developed no dangerous symptoms and became normal in 10 days. On the 12th day 0.5 c.c. of the culture was given intravenously. This caused rather severe symptoms, but the animal was completely recovered in 5 days.

Rabbit 176 received 0.1 c.c. of the culture and 5 c.c. of the serum. This rabbit developed no symptoms at all. On the 12th day it was given 0.5 c.c. of the culture intravenously. This secondary dose also produced no ill effects, the rabbit being completely normal in 3 days. It had gained 43 grams in weight during the experiment.

Rabbit 179 received 0.1 c.c. of the culture and 10 c.c. of the serum. As no symptoms were produced, the rabbit, 12 days later, received 0.5 c.c. of the culture. No ill effects were produced.

A pregnant guinea-pig received 1 c.c. of a 24-hour mixed-broth culture of B. abortivo-equinus subcutaneously, and 5 c.c. of the hyperimmune blood "5/26" also subcutaneously. This guinea-pig aborted 4 fetuses 17 days after the inoculation—12 days longer than is usually required for an abortion after such a dose.

From these experiments, it looked as if the hyperimmune serum were producing marked effects, and the following test was made on a pregnant mare.

A preliminary blood test showed that the mare had no immunity to the disease. The animal received, intrajugularly, 1 c.c. of a mixed culture washed from an agar slant and diluted in 5 c.c. of normal salt solution, and 200 c.c. of the hyperimmune blood "5/26," subcutaneously. For the first 2½ days after inoculation the animal did not eat. After this no symptoms were observed, except that the temperature rose to 104 F. 2 days after inoculation. On the 13th day, a well-developed fetus was discharged and B. abortivo equinus was recovered from its organs. The hyperimmune serum had not protected from an aborting dose of the organism.

Among the questions now being studied in this laboratory are (1) whether a larger dose of the serum will protect; (2) whether the serum will prove of value in a natural infection; (3) whether the serum can be produced in sufficient quantities to prove of practical value; (4) whether a vaccine will prove of value in building up sufficient immunity to protect against the disease.

SUMMARY

A hyperimmune serum for infectious abortion in mares can be produced that has marked bacteriolytic properties in vitro.

This serum protects rabbits from the lethal dose of the organism. In one instance it protected from 5 times the lethal dose.

The serum lengthened the time for abortion in one guinea-pig 12 days.

The serum did not protect a mare from an artificial infection. The amount of the organism given, inoculated directly into the blood, was vastly more than could be gotten in a natural infection. The serum may prove of value in a stud where the infection is known to exist.